EXPERIMENTAL STUDY OF THE IMMUNOGENIC PROPERTIES OF ASSOCIATED ANAEROBIC TOXOIDS

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EXPERIMENTAL STUDY OF THE IMMUNOGENIC PROPERTIES OF ASSOCIATED ANAEROBIC TOXOIDS

Communication V

Immunogenic Properties of Combined Polytoxoid in Primary Immunization of Animals

[Following is a translation of an article by G. V. Vygodchikov, A. A. Vorob'yev, I. A. Larina, A. P. Labinskiy, V. D. Gekker, V. M. Shevelev, and N. S. Sergeyeva, Gamaleya Institute of Epidemiology and Microbiology, USSR Academy of Medical Sciences, published in the Russian-language periodical Zhurnal mikrobiologii, epidemiologii i immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology) No 10, 1963, pages 51 - 58. The article was received 12 Dec 1962. Translation performed by Sp/6 Charles T. Ostertag Jr.]

Problems dealing with the study of the immunological effectiveness of complex vaccines which are made up of various combinations of anaerobic and intestinal antigens have been elucidated in a small number of works (Krestovnikova et al., 1956; Vorob'yev and Bron, 1957; Kondrat'yev, 1958; Ponomarev et al., 1958). Our first attempts at creating such associated vaccines (pentatoxoid + intestinal antigens, hexatoxoid + intestinal antigens) showed that the dysenteric component is immunologically non-effective and, therefore, it isn't advisable to include it in the complex. It was also established that the intestinal components (typhoid - paratyphoid) are more weakly antigenic in comparison with anaerobic toxoids and if there is an insufficient dose of them in the complex then immunological competition is possible.[1]

[1] Communication I, J. of Microbiology, No 1, 1961; Communication II, Ibid, No 7, 1961; Communication III, Ibid, No 8, 1962; Communication IV, Ibid, No 1, 1963.

In the present work, facts are presented on the study of an associated vaccine (combined polytoxoid) made up of 10 components [gangrenous (perfringens and oedematiens), tetanus, botulism, (A, B, C, D, and E)] toxoids and two intestinal antigens (typhoid O and paratyphoid B). An octatoxoid which contained in 1 ml the same doses of anaerobic toxoids without the intestinal antigens was the control for this preparation. White mice, guinea pigs, rabbits and monkeys were used for verifying the immunological effectiveness of the complex preparations. A 1.5 ml mixture of octatoxoid with intestinal antigens contained the following toxoids: Perfringens -- 40 EC[2], oedematiens -- 40 EC, tetanus -- 10 EC, botulism A -- 100 EC, B -- 50 EC, botulism C, D, and E -- 25 EC each; and the antigens: Typhoid Vi antigen -- 0.1 mg, O-antigen -- 0.05 mg, and paratyphoid B -- 0.1 mg.

[2] For all the toxoids 1 EC corresponded to 1 AE of specific antiserum [AE -- antigen unit].

All the antigens were sorbed on aluminum hydroxide with a calculation that one immunizing dose (1.5 ml) contained 4.7 mg of Al_2C_3. The preparation was introduced subcutaneously since during intraperitoneal immunization a greater mortality of mice was observed.

Guinea pigs with a weight of 300 - 350 grams were given a single subcutaneous immunization with an inoculation dose of combined polytoxoid (1.5 ml) and octatoxoid (1 ml). In 25 days following the immunization blood was taken from the animals for determining the antibody titer and in 30 days the intensity of immunity was determined by introducing various doses of toxins.

As is apparent from table 1, the levels of antitoxins in guinea pigs inoculated with the combined polytoxoid and octatoxoid were approximately the same. Also, these animals didn't differ in their immunity to the administration of toxins. The only point worth noting was the lower survival rate of guinea pigs immunized with the combined polytoxoid following the administration of gangrenous toxins (oedematiens, perfringens). It follows to note also the very satisfactory immunity of the animals against botulism toxin Type E, in spite of their lack of specific titers of antitoxin in the blood.

Rabbits were given a double immunization with an interval of 45 days. In each group of five animals the same doses were used during the first and second injections. The level of antibodies in the rabbits was determined 45 days after the first and 15 days after the second immunization. The mixture of sera was titrated by groups.

An analysis of the data in table 2 showed that a single subcutaneous administration of a whole dose of combined polytoxoid (1.5 ml) caused an accumulation of antitoxins on a specific level in relation to all the antigens. Lowering the dose of antigens by 10 and 20 times was accompanied by a lowering of the antitoxin titers. However, after a double immunization of even 1/20 of a dose the titers of antibodies were sufficiently high. Antitoxic immunity in rabbits following the administration of the combined preparation was in several cases even higher than following immunization with the

octatoxoid (whole dose equal to 1 ml). After the second inoculation, this difference leveled off.

We also immunized two groups of monkeys with the preparations being tested (six animals in each group). Octatoxoid was administered in an amount of 1 ml, combined polytoxoid -- 1.5 ml. In the present work data is presented on the initial immunization since the high titers obtained after the second immunization evened out the difference in the effectiveness of the antigens. Blood was drawn 25 days after the first immunization. As is apparent from table 3, high antibody titers were observed more often in the monkeys immunized with combined polytoxoid.

The immunogenic activity of intestinal antigens in the combined polytoxoid was studied in white mice weighing 15 - 17 grams. They were given a single subcutaneous immunization of the following: a) a mixture of antigens of the intestinal group of bacteria sorbed by aluminum hydroxide; b) associated vaccine made up of antigens of the intestinal group of bacteria and hexatoxoid; c) combined polytoxoid. All the animals were immunized with half of the inoculative dose. In 20 days following the immunization, the animals were infected with 1 and 2 Dcl of a typhoid culture (strain Ty_2 4446).

As is apparent from the data presented in table 4, the typhoid component of associated vaccine possessed very high immunogenic properties. The group of mice immunized only with the antigens of the intestinal group of bacteria were protected following infection with a typhoid culture to the same degree as animals of other groups vaccinated with intestinal vaccine and a complex of anaerobic toxoids.

Together with the study of the active immunity developing in response to the introduction of associated vaccine, we also determined the passive immunity. With this aim we subjected rabbits to a single and double subcutaneous immunization with various doses of the preparation -- 1, 1/10, and 1/20 of an inoculative dose. The preventive properties of their sera was determined 45 days after a single immunization and 15 days after a double immunization. Serum in various doses was introduced subcutaneously to mice. After 18 hours they were infected intraperitoneally with 1 Dcl of typhoid culture (strain Ty_2 4446).

From table 5 it is apparent that the antisera possessed high preventive properties; doses of serum from 0.2 to 0.125 ml protected almost 100% of the animals used in the experiment following infection with 1 Dcl of a typhoid culture.

We compared the immunogenic properties of dry and liquid combined polytoxoid. We obtained the dry sorbed combined polytoxoid by means of lyophilic drying of the liquid preparation with the addition, in the capacity of a

filler, of saccharose and gelatin (Saltykov et al., 1961). The experiment was set up in the same manner as mentioned above. The animals were immunized with half of a test dose of the preparation. As is apparent from table 6, the immunogenic properties of typhoid and paratyphoid B components in dry and liquid vaccines were completely similar.

CONCLUSIONS

1. In experiments on various species of animals (white mice, pigs, rabbits, monkeys) it has been shown that multi-component preparations against anaerobic and intestinal infections possessed expressed antigenic and immunogenic properties.

2. In experimental animals the initial immunization with associated vaccine caused a sufficiently intense antitoxic immunity in relation to wound infections and botulism.

3. Typhoid and paratyphoid B components, included in an associated vaccine, possessed sufficiently expressed antigenic and immunogenic properties.

4. In experiments on monkeys and rabbits, the stimulating effect has been demonstrated of antigens derived from the intestinal-typhoid group of bacteria on the immunological effectiveness of toxoids of anaerobic infections. In experiments on guinea pigs, a certain oppression of gangrenous antigens was exposed following immunization with a combined preparation.

5. In studying the immunogenic properties of associated preparations it is advisable to conduct experiments on various species of animals.

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The following English summary appears with the Russian article.

The paper deals with experimental data on the immunogenicity of a complex associated preparation, consisting of 8 anaerobic toxins and complete antigens of typhoid and paratyphoid B bacilli. The efficacy of the associated preparation in respect to all the antigens contained was shown in primary immunization of animals.

Table 1

Level of antitoxins in the blood and the intensity of immunity to toxins in guinea pigs after a single administration of one inoculation dose of combined polytoxoid (1.5 ml) and octatoxoid (1 ml).

Component of associated preparation (toxoid)	Results of immunization					
	Combined polytoxoid			Octatoxoid		
	Titer (antigen units/ml)	Infection		Titer (antigen units/ml)	Infection	
		Dose of toxin (Dcl)	Result		Dose of toxin (Dcl)	Result
Tetanus	$\leqslant$ 0.5	500	2/3	$\geqslant$ 0.6	500	3/3
		1000	2/3		1000	3/3
		2000	3/3		2000	2/3
Perfringens	$<$ 0.1	1	1/2	$>$0.1 $<$ 0.3	1	2/2
		2	0/3		2	3/3
		4	2/3		4	2/3
		10	0/2		10	0/2
Oedematiens	2	100	2/3	2	100	3/3
		200	0/3		200	3/3
		500	0/3		500	1/3
Botulism A	1	500	3/3	3	500	3/3
		1000	4/4		1000	4/4
		2000	3/3		2000	3/3
B	2	250	3/3	$<$ 10	250	3/3
		500	3/3		500	4/4
		1000	2/3		1000	3/3
C	2	125	3/3	2	125	3/3
		250	4/4		250	4/4
		500	3/3		500	3/3
D	1	25	3/3	2	25	3/3
		20	4/4		50	4/4
		100	3/3		100	3/3
E	$<$ 0.05	250	2/3	$<$ 0.05	250	2/3
		500	3/4		500	3/3
		1000	2/3		1000	1/2

Numerator -- number of surviving animals; denominator -- number of animals in the experiment.

Table 2

Name of preparation	Dose of preparation for immunization	Content of antitoxin in serum (in antigen units/ml) 45 days after first immunization: Botulism A	B	C	D	E	Gangrenous tetanus	Gangrenous perfringens	Gangrenous oedematiens	15 days after second immunization: Botulism A	B	C	D	E	Gangrenous tetanus	Gangrenous perfringens	Gangrenous oedematiens
Octatoxoid	1/20 full dose	<0.05	>0.05 <0.1	0.1	<0.1	0.1	<0.05	0.05	>0.1	1.0	>1 <3	>20 <40	>20 <40	>10 <30	<1.0	0.3 <1.0	10
Combined polytoxoid		<0.05	<0.3	1.0	1.0	<0.1	<0.05	0.05	>0.1	>3 <10	>1 <3	>20 <40	>20 <40	<10	>0.3 <1.0	>0.3 <1.0	>5 <10
Octatoxoid	1/10 full dose	<0.05	<0.1	>1.0 <3.0	>1.0 <3.0	0.5	<0.05	>0.1	>0.1	3	3	>20 <40	>20	>10 <30	1.0	>0.3 <1.0	>10
Combined polytoxoid		<0.05	0.1	1.0	0.3	0.3	<0.05	0.05	>0.1	3	>1 <3	<20	>10 <20	>5 <10	0,1	>0.3 <1.0	>10
Octatoxoid	Full dose	<0.05	<0.3	3.0	3.0	1.0	0.3	<0.05	>0.3	<10	>3 <10	>60	>20	>20 <40	<5.0	>1.0 <3.0	>20
Combined polytoxoid		<0.1	>0.5 <1.0	>5.0 <10.0	>10.0	1.0	>0.5 <3.0	>0.1 <0.3	>0.3	10	>3 <10	>40 <80	20	>10 <20	3.0	>1.0 <3.0	>20

Levels of antitoxins in the blood of rabbits inoculated with various doses of combined polytoxoid and octatoxoid

Table 3

Levels of antitoxins in monkeys after a single immunization with combined polytoxoid and octatoxoid

Name of monkey	Type of antigen	Titer of antibodies 25 days after immunization (in antigen units/ml)							
		Gangrenous			Botulism				
		perfringens	oedematiens	tetanus	A	B	C	D	E
Sparzha	Combined polytoxoid 1.5 ml	>0.1	>0.1 <0.5	≤0.1	>0.05	>1.0	> 0.1 < 0.5	>0.1	>0.05 <0.1
Sidney		<0.1	>0.1 <0.5	>0.1	≥0.05	>0.25	>0.1	<0.1	>0.5
Sliva		<0.1	> 0.5	>0.1	≥0.05	>0.25 <1.0	<0.5 ≤0.5	>0.5 <1.0	≥0.1
Volshebnitsa		<0.1	>0.1	>0.1	>0.25	>0.25	≤ 0.1	>0.5	>0.1 <0.5
Zel'va		<0.1	>0.1 <0.5	≥0.1	≥0.05	≥1	>0.5	≥0.1	<0.05
Ako		<0.1	≥0.1	>0.1	>0.05 <0.25	>0.25 <1	>0.1 <0.5	>0.5	<0.05
Mokritsa		<0.1	>0.1 <0.5	≤0.1	>0.05 <0.25	>0.05 <0.25	≥ 0.1	>0.05 <0.1	>0.1 <0.5
Domb		<0.1	≥0.1	≤0.1	≥0.05	>0.05 <0.25	≤ 0.1	<0.1	>0.05 <0.1
Zhirdon	Octatoxoid 1 ml	<0.1	≥0.1	≤0.1	≥0.05	≤1.0	≤ 0.1	<0.1	>0.05 <1.0
Ems		<0.1	<0.1	≤0.1	≤0.05	≥1.0	≥0.1	≥0.1	<0.05
Linda		<0.1	≤0.1	≤0.1	≥0.05	≥1.0	≤0.1	<0.1	<0.05
Taksik		<0.1	>0.1 <0.5	<0.1	>0.05 <0.1	>0.05 <0.25	≥0.5	<0.05	<0.05

Immunogenic properties of the typhoid component in an associated vaccine against anaerobic and intestinal infections

Table 4

Composition of preparation	Infection dose of Ty_2 (in Dcl)	Result		Survival rate (%)	Average error
		Infected	Survived		
Antigens of the intestinal-typhoid group of bacteria	1	20	19	95	± 4.8
	2	13	12	92.2	± 7.1
Antigens of the intestinal-typhoid group of bacteria + hexatoxoid	1	20	17	85	± 7.9
	2	13	12	92.2	± 7.4
Antigens of the intestinal-typhoid group of bacteria + octatoxoid	1	20	17	85	± 7.9
	2	13	11	84.8	± 11.6
Control culture of Ty_2	100 million	10	0		
	50 "	10	0		
	25 "	10	7		

Table 5

Preventive properties of sera from rabbits immunized with combined polytoxoid

Frequency of immunization	Preparation and dose of immunization	Period when blood taken (days)	Infection dose (in millions)	Dose of serum (in ml)	Result of experiment: Survived	Result of experiment: Infected
Single	Antigens + octatoxoid 1 inoculation dose	45	50	0.2	6	6
				0.1	6	6
				0.05	11	12
				0.025	5	6
				0.0125	5	6
	Antigens + octatoxoid 1/10 inoculation dose	45	50	0.2	6	6
				0.1	6	6
				0.05	11	12
				0.025	6	6
				0.0125	5	6
	Antigens + octatoxoid 1/20 inoculation dose	45	50	0.2	6	6
				0.1	6	6
				0.05	11	13
				0.025	6	6
				0.0125	5	6
	Control of culture		50	-	1	15
			25	-	4	10
Double	Antigens + octatoxoid 1 inoculation dose	15	50	0.1	4	5
				0.05	5	5
				0.025	5	5
	Antigens + octatoxoid 1/10 inoculation dose	15	50	0.1	5	5
				0.05	5	5
				0.025	4	5
	Antigens + octatoxoid 1/20 inoculation dose	15	50	0.1	5	5
				0.05	5	5
				0.025	5	5
	Control of culture		50	-	0	10
			25	-	2	5

Comparison of immunogenic properties of intestinal antigens in a dry and liquid combined polytoxoid

Composition of preparation	State of preparation	infection Culture	Dose	Result Survived	Result Infected	Survival rate %	Average error
Typhoid O-antigen -- 0.05 mg, typhoid Vi-antigen -- 0.1 mg, paratyphoid B-antigen -- 0.1 mg, octatoxoid	Dry	Typhoid	1 Dcl	20	20	100	
			2 Dcl	15	20	75	± 9.6
		Paratyphoid B	1 Dcl	14	20	70	± 10.2
	Liquid	Typhoid	1 Dcl	19	20	95	± 4.87
			2 Dcl	15	20	75	± 9.6
		Paratyphoid B	1 Dcl	14	20	70	± 10.2
Control of culture	—	Typhoid	100 mil.	0	10		
			50 mil.	0	10		
			25 mil.	2	10		
	—	Paratyphoid B	50 mil.	0	5		
			25 mil.	3	5		

Table 6